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(54) Title: NF-KB INHIBITORS

(57) Abstract: The present invention provides a specific compound and methods for treating diseases related to the inhibition of IKK- β phosphorylation of Ik.

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NF- κ B Inhibitors**FIELD OF THE INVENTION**

This invention relates in general to a method of inhibiting pathological activation of the transcription factor NF- κ B (nuclear factor- κ B) using
5 aminothiophene compounds. Such methods are particularly useful for treating diseases in which activation of NF- κ B is implicated. More specifically, these methods may be used for inhibiting IKK- β (I κ B kinase- β , also known as IKK-2) phosphorylation of I κ B (inhibitory protein κ B)-which prevents subsequent degradation and activation of NF- κ B dimers. Such methods are useful in the
10 treatment of a variety of diseases associated with NF- κ B activation including inflammatory and tissue repair disorders; particularly rheumatoid arthritis, inflammatory bowel disease, asthma and COPD (chronic obstructive pulmonary disease) osteoarthritis; osteoporosis and fibrotic diseases; dermatosis, including psoriasis, atopic dermatitis and ultraviolet radiation (UV)-induced skin damage;
15 autoimmune diseases including systemic lupus erythematosus, multiple sclerosis, psoriatic arthritis, ankylosing spondylitis, tissue and organ rejection, Alzheimer's disease, stroke, atherosclerosis, restenosis, diabetes, glomerulonephritis, cancer, including Hodgkins disease, cachexia, inflammation associated with infection and certain viral infections, including acquired immune deficiency syndrome (AIDS), adult
20 respiratory distress syndrome, and Ataxia Telangiectasia.

BACKGROUND OF THE INVENTION

Recent advances in scientific understanding of the mediators involved in acute and chronic inflammatory diseases and cancer have led to new strategies in the
25 search for effective therapeutics. Traditional approaches include direct target intervention such as the use of specific antibodies, receptor antagonists, or enzyme inhibitors. Recent breakthroughs in the elucidation of regulatory mechanisms involved in the transcription and translation of a variety of mediators have led to increased interest in therapeutic approaches directed at the level of gene
30 transcription.

Nuclear factor κ B (NF- κ B) belongs to a family of closely related dimeric transcription factor complexes composed of various combinations of the Rel/NF- κ B family of polypeptides. The family consists of five individual gene products in mammals, RelA (p65), NF- κ B1 (p50/ p105), NF- κ B2 (p49/ p100), c-Rel, and RelB, all of which can form hetero- or homodimers. These proteins share a highly homologous 300 amino acid "Rel homology domain" which contains the DNA binding and dimerization domains. At the extreme C-terminus of the Rel homology domain is a nuclear translocation sequence important in the transport of NF- κ B from the cytoplasm to the nucleus. In addition, p65 and cRel possess potent transactivation domains at their C-terminal ends.

The activity of NF- κ B is regulated by its interaction with a member of the inhibitor I κ B family of proteins. This interaction effectively blocks the nuclear localization sequence on the NF- κ B proteins, thus preventing migration of the dimer to the nucleus. A wide variety of stimuli activate NF- κ B through what are likely to be multiple signal transduction pathways. Included are bacterial products (LPS), some viruses (HIV-1, HTLV-1), inflammatory cytokines (TNF α , IL-1), environmental and oxidative stress and DNA damaging agents. Apparently common to all stimuli however, is the phosphorylation and subsequent degradation of I κ B. I κ B is phosphorylated on two N-terminal serines by the recently identified I κ B kinases (IKK- α and IKK- β). Site-directed mutagenesis studies indicate that these phosphorylations are critical for the subsequent activation of NF- κ B in that once phosphorylated the protein is flagged for degradation via the ubiquitin-proteasome pathway. Free from I κ B, the active NF- κ B complexes are able to translocate to the nucleus where they bind in a selective manner to preferred gene-specific enhancer sequences. Included in the genes regulated by NF- κ B are a number of cytokines and chemokines, cell adhesion molecules, acute phase proteins, immunoregulatory proteins, eicosanoid metabolizing enzymes and anti-apoptotic genes.

It is well-known that NF- κ B plays a key role in the regulated expression of a large number of pro-inflammatory mediators including cytokines such as TNF, IL-1 β , IL-6 and IL-8, cell adhesion molecules, such as ICAM and VCAM, and

inducible nitric oxide synthase (iNOS). Such mediators are known to play a role in the recruitment of leukocytes at sites of inflammation and in the case of iNOS, may lead to organ destruction in some inflammatory and autoimmune diseases.

The importance of NF- κ B in inflammatory disorders is further strengthened by studies of airway inflammation including asthma, in which NF- κ B has been shown to be activated. This activation may underlie the increased cytokine production and leukocyte infiltration characteristic of these disorders. In addition, inhaled steroids are known to reduce airway hyperresponsiveness and suppress the inflammatory response in asthmatic airways. In light of the recent findings with regard to glucocorticoid inhibition of NF- κ B, one may speculate that these effects are mediated through an inhibition of NF- κ B.

Further evidence for a role of NF- κ B in inflammatory disorders comes from studies of rheumatoid synovium. Although NF- κ B is normally present as an inactive cytoplasmic complex, recent immunohistochemical studies have indicated that NF- κ B is present in the nuclei, and hence active, in the cells comprising rheumatoid synovium. Furthermore, NF- κ B has been shown to be activated in human synovial cells in response to stimulation with TNF- α or IL-1 β . Such a distribution may be the underlying mechanism for the increased cytokine and eicosanoid production characteristic of this tissue. See Roshak, A. K., et al., *J. Biol. Chem.*, **271**, 31496-31501 (1996). Expression of IKK- β has been shown in synoviocytes of rheumatoid arthritis patients and gene transfer studies have demonstrated the central role of IKK- β in stimulated inflammatory mediator production in these cells. See Aupperle et al. *J. Immunology* 1999. 163:427-433 and Aupperle et al. *J. Immunology* 2001;166:2705-11. More recently, the intra-articular administration of a wild type IKK- β adenoviral construct was shown to cause paw swelling while intra-articular administration of dominant-negative IKK- β inhibited adjuvant-induced arthritis in rat. See Tak et al. *Arthritis and Rheumatism* 2001; 44:1897-1907.

The NF- κ B/Rel and I κ B proteins are also likely to play a key role in neoplastic transformation and metastasis. Family members are associated with cell transformation *in vitro* and *in vivo* as a result of overexpression, gene amplification,

gene rearrangements or translocations. In addition, rearrangement and/or amplification of the genes encoding these proteins are seen in 20-25% of certain human lymphoid tumors. Further, NF- κ B is activated by oncogenic ras, the most common defect in human tumors and blockade of NF- κ B activation inhibits ras mediated cell transformation. In addition, a role for NF- κ B in the regulation of apoptosis has been reported, strengthening the role of this transcription factor in the regulation of tumor cell proliferation. TNF, ionizing radiation and DNA damaging agents have all been shown to activate NF- κ B which in turn leads to the upregulated expression of several anti-apoptotic proteins. Conversely, inhibition of NF- κ B has been shown to enhance apoptotic-killing by these agents in several tumor cell types. As this likely represents a major mechanism of tumor cell resistance to chemotherapy, inhibitors of NF- κ B activation may be useful chemotherapeutic agents as either single agents or adjunct therapy. Recent reports have implicated NF- κ B as an inhibitor of skeletal cell differentiation as well as a regulator of cytokine-induced muscle wasting (Guttridge et al. *Science*; 2000; 289: 2363-2365.) further supporting the potential of NF- κ B inhibitors as novel cancer therapies.

Several NF- κ B inhibitors are described in C. Wahl, et al. *J. Clin. Invest.* 101(5), 1163-1174 (1998), R. W. Sullivan, et al. *J. Med. Chem.* 41, 413-419 (1998), J. W. Pierce, et al. *J. Biol. Chem.* 272, 21096-21103 (1997)

The marine natural product hymenialdisine is known to inhibit NF- κ B. Roshak, A., et al., *JPET*, **283**, 955-961 (1997). Breton, J. J and Chabot-Fletcher, M. C., *JPET*, **282**, 459-466 (1997).

Additionally, patent applications have disclosed inhibitors of IKK-2 or the IKK complex, see WO 200246171, , WO 200198290 , WO 200168648, EP 1134221, DE 19807993, EP 1209158, WO 200130774, DE 19928424, WO 200100610, WO 200224679, WO 200224693, WO 200244153, WO 200228860, WO 200260386, WO 200241843, and WO 200262804. Patent applications including ureidothiophenes include WO 200158890 and WO200230353. PS-1145 was shown to be an IKK-2 inhibitor see *JBC* 2002, 19, 16639-16647 Natural products such as staurosporine, quercetin, K252a and K252b have been shown to be

IKK- β inhibitors, see Peet, G. W. and Li, J. J. *Biol. Chem.*, 274, 32655-32661 (1999) and Wisniewski, D., et al., *Analytical Biochem.* 274, 220-228 (1999).

SUMMARY OF THE INVENTION

The present invention involves a novel compound and methods of using it
5 for inhibiting the activation transcription factor NF- κ B.

An object of the present invention is to provide a method for treating diseases which may be therapeutically modified by altering the activity of transcription factor NF- κ B.

Accordingly, in the first aspect, this invention provides a pharmaceutical
10 composition comprising the present compound.

In another aspect, this invention provides a method of treating diseases in which the disease pathology may be therapeutically modified by inhibiting phosphorylation and subsequent degradation of I κ B by IKK- β .

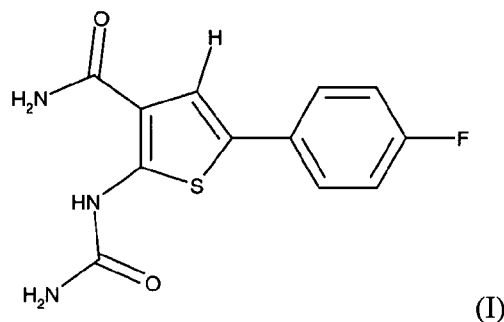
In still another aspect, this invention provides a method of treating diseases
15 in which the disease pathology may be therapeutically modified by inhibiting pathological activation of NF- κ B.

In a particular aspect, this invention provides methods for treating a variety of diseases associated with NF- κ B activation including inflammatory and tissue repair disorders, particularly rheumatoid arthritis, inflammatory bowel disease,
20 asthma and COPD (chronic obstructive pulmonary disease) osteoarthritis, osteoporosis and fibrotic diseases, dermatosis, including psoriasis, atopic dermatitis and ultraviolet radiation (UV)-induced skin damage; autoimmune diseases including systemic lupus erythematosus, multiple sclerosis, psoriatic arthritis, ankylosing spondylitis, tissue and organ rejection, Alzheimer's disease, stroke, atherosclerosis,
25 restenosis, diabetes, glomerulonephritis, cancer, including Hodgkins disease, cachexia, inflammation associated with infection and certain viral infections, including acquired immune deficiency syndrome (AIDS), adult respiratory distress syndrome and Ataxia Telangiectasia

DETAILED DESCRIPTION OF THE INVENTION

The compound of the present invention is shown in Formula (I) herein below:

5



(I)

The compound of Formula 1 can also be represented as: 5-(4-fluoro-phenyl)-2-ureido-thiophene-3-carboxylic acid amide

10 The present invention further provides a preferred method of treatment of diseases associated with NF- κ B activation, comprising administering to a subject in need thereof.

15 This invention provides methods for treating a variety of diseases associated with NF- κ B activation including inflammatory and tissue repair disorders; particularly rheumatoid arthritis, inflammatory bowel disease, asthma and COPD (chronic obstructive pulmonary disease) osteoarthritis, osteoporosis and fibrotic diseases; dermatosis, including psoriasis, atopic dermatitis and ultraviolet radiation (UV)-induced skin damage; autoimmune diseases including systemic lupus erythematosus, 20 multiple sclerosis, psoriatic arthritis, ankylosing spondylitis, tissue and organ rejection, Alzheimer's disease, stroke, atherosclerosis, restenosis, diabetes, glomerulonephritis, cancer, including Hodgkins disease, cachexia, inflammation associated with infection and certain viral infections, including acquired immune deficiency syndrome (AIDS), adult respiratory distress syndrome, and Ataxia Telangiectasia.

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DEFINITIONS

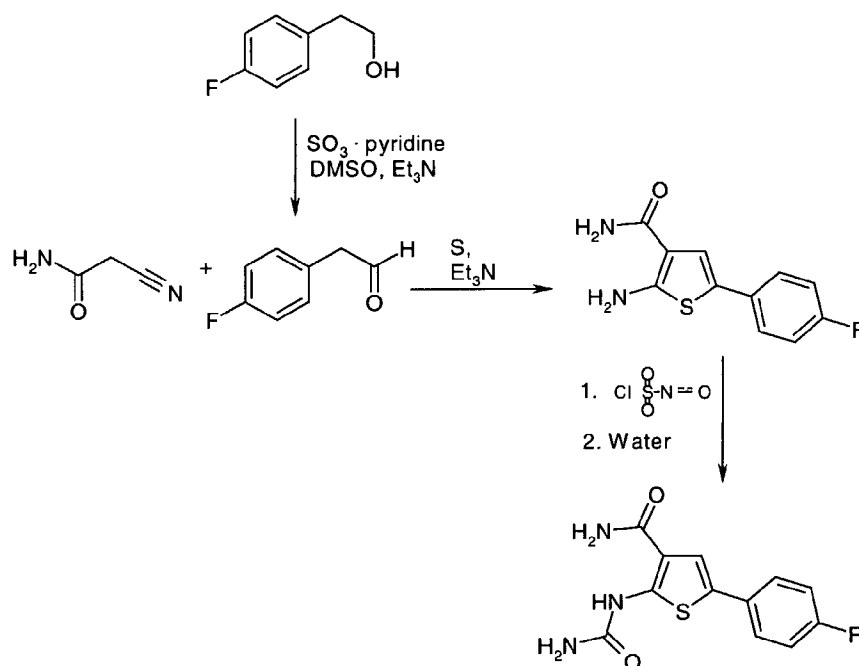
The present invention includes all hydrates, solvates, complexes and prodrugs of the compound of this invention. Prodrugs are any covalently bonded compounds, which release the active parent drug according to Formula I *in vivo*.

5

METHODS OF PREPARATION

The compound of Formula I of the present invention may be conveniently prepared by the methods set forth in Scheme 1 below.

Scheme 1



10

The starting materials used herein are commercially available or are prepared by routine methods well known to those of ordinary skill in the art and can be found in standard reference books, such as the COMPENDIUM OF ORGANIC SYNTHETIC METHODS, Vol. I-VI (published by Wiley-Interscience).

- 5 This invention provides a pharmaceutical composition, which comprises a compound according to Formula I and a pharmaceutically acceptable carrier, diluent or excipient. Accordingly, the compound of Formula I may be used in the manufacture of a medicament. Pharmaceutical compositions of the compound of Formula I prepared as hereinbefore described may be formulated as solutions or
- 10 lyophilized powders for parenteral administration. Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. The liquid formulation may be a buffered, isotonic, aqueous solution. Examples of suitable diluents are normal isotonic saline solution, standard 5% dextrose in water or buffered sodium or ammonium acetate solution. Such
- 15 formulation is especially suitable for parenteral administration, but may also be used for oral administration or contained in a metered dose inhaler or nebulizer for insufflation. It may be desirable to add excipients such as polyvinylpyrrolidone, gelatin, hydroxy cellulose, acacia, polyethylene glycol, mannitol, sodium chloride or sodium citrate.
- 20 Alternately, this compound may be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, magnesium stearate or stearic acid, talc, pectin, acacia,
- 25 agar or gelatin. Liquid carriers include syrup, peanut oil, olive oil, saline and water. The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax. The amount of solid carrier varies but, preferably, will be between about 20 mg to about 1 g per dosage unit. The pharmaceutical preparations are made following the conventional
- 30 techniques of pharmacy involving milling, mixing, granulating, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin

capsule forms. When a liquid carrier is used, the preparation will be in the form of a syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly p.o. or filled into a soft gelatin capsule.

Typical compositions for inhalation are in the form of a dry powder, solution, suspension or emulsion. Administration may, for example, be by dry powder inhaler (such as unit dose or multi-dose inhaler, e.g. as described in US Patent 5590645 or by nebulisation or in the form of a pressurized aerosol. Dry powder compositions typically employ a carrier such as lactose, trehalose or starch. Compositions for nebulisation typically employ water as vehicle. Pressurized aerosols typically employ a propellant such as dichlorodifluoromethane, trichlorofluoromethane or, more preferably, 1,1,1,2-tetrafluoroethane, 1,1,1,2,3,3,3-heptafluoro-n-propane or mixtures thereof. Pressurized aerosol formulations may be in the form of a solution (perhaps employing a solubilising agent such as ethanol) or a suspension which may be excipient free or employ excipients including surfactants and/or co-solvents (e.g. ethanol). In dry powder compositions and suspension aerosol compositions the active ingredient will preferably be of a size suitable for inhalation (typically having mass median diameter (MMD) less than 20 microns e.g. 1-10 especially 1-5 microns). Size reduction of the active ingredient may be necessary e.g. by micronisation.

Pressurized aerosol compositions will generally be filled into canisters fitted with a valve, especially a metering valve. Canisters may optionally be coated with a plastics material e.g. a fluorocarbon polymer as described in WO96/32150. Canisters will be fitted into an actuator adapted for buccal delivery.

Typical compositions for nasal delivery include those mentioned above for inhalation and further include non-pressurized compositions in the form of a solution or suspension in an inert vehicle such as water optionally in combination with conventional excipients such as buffers, anti-microbials, tonicity modifying agents and viscosity modifying agents which may be administered by nasal pump.

For rectal administration, the compound of this invention may also be combined with excipients such as cocoa butter, glycerin, gelatin or polyethylene glycols and molded into a suppository.

The methods of the present invention include topical, inhaled and intracolonic administration of the compound of Formula I. By topical administration is meant non-systemic administration, including the application of a compound of the invention externally to the epidermis, to the buccal cavity and
5 instillation of such a compound into the ear, eye and nose, wherein the compound does not significantly enter the blood stream. By systemic administration is meant oral, intravenous, intraperitoneal and intramuscular administration. The amount of a compound of the invention (hereinafter referred to as the active ingredient) required for therapeutic or prophylactic effect upon topical administration will, of course,
10 vary with the compound chosen, the nature and severity of the condition being treated and the animal undergoing treatment, and is ultimately at the discretion of the physician

While it is possible for an active ingredient to be administered alone as the raw chemical, it is preferable to present it as a pharmaceutical formulation. The
15 active ingredient may comprise, for topical administration, from 0.01 to 5.0 wt%.of the formulation.

The topical formulations of the present invention, both for veterinary and for human medical use, comprise an active ingredient together with one or more acceptable carriers therefor and optionally any other therapeutic ingredients. The
20 carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required such as: liniments, lotions, creams, ointments or pastes, and drops
25 suitable for administration to the eye, ear or nose.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active
30 agent. The resulting solution may then be clarified by filtration, transferred to a suitable container, which is then sealed and sterilized by autoclaving, or maintaining

at 90-100 C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap, a mucilage, an oil of natural origin such as almond, corn, arachis, castor or olive oil, wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogols. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surface active agent such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as siliceous silicas, and other ingredients such as lanolin, may also be included.

UTILITY OF THE PRESENT INVENTION

The compound of Formula I is useful as an inhibitor of the IKK-beta kinase phosphorylation of I κ B and as such is an inhibitor of NF- κ B activation. The present

method utilizes compositions and formulations of said compound, including pharmaceutical compositions and formulations of said compound.

The present invention particularly provides methods of treatment of diseases associated with inappropriate NF- κ B activation, which methods comprise
5 administering to an animal, particularly a mammal, most particularly a human in need thereof a compound of Formula I. The present invention particularly provides methods for treating inflammatory and tissue repair disorders, particularly rheumatoid arthritis, inflammatory bowel disease, asthma and COPD (chronic obstructive pulmonary disease) osteoarthritis, osteoporosis and fibrotic diseases; dermatosis,
10 including psoriasis, atopic dermatitis and ultraviolet radiation (UV)-induced skin damage, autoimmune diseases including systemic lupus erythematosus, multiple sclerosis, psoriatic arthritis, ankylosing spondylitis, tissue and organ rejection, Alzheimer's disease, stroke, atherosclerosis, restenosis, diabetes, glomerulonephritis, cancer, including Hodgkins disease, cachexia, inflammation associated with infection
15 and certain viral infections, including acquired immune deficiency syndrome (AIDS), adult respiratory distress syndrome and Ataxia Telangiectasia.

For acute therapy, parenteral administration of the compound of Formula I is useful. An intravenous infusion of the compound in 5% dextrose in water or normal saline, or a similar formulation with suitable excipients, is most effective, although
20 an intramuscular bolus injection is also useful. Typically, the parenteral dose will be about 0.01 to about 50 mg/kg; preferably between 0.1 and 20 mg/kg, in a manner to maintain the concentration of drug in the plasma at a concentration effective to inhibit IKK-beta and therefore activation of NF- κ B. The compound is administered one to four times daily at a level to achieve a total daily dose of about 0.4 to about
25 80 mg/kg/day. The precise amount of the compound used in the present method which is therapeutically effective, and the route by which such compound is best administered, is readily determined by one of ordinary skill in the art by comparing the blood level of the agent to the concentration required to have a therapeutic effect.

The compound of Formula I may also be administered orally to the patient,
30 in a manner such that the concentration of drug is sufficient to inhibit IKK-beta and therefore activation of NF- κ B or to achieve any other therapeutic indication as

disclosed herein. Typically, a pharmaceutical composition containing the compound is administered at an oral dose of between about 0.1 to about 50 mg/kg in a manner consistent with the condition of the patient. Preferably the oral dose would be about 0.5 to about 20 mg/kg.

5 The compound of Formula I may also be administered topically to the patient, in a manner such that the concentration of drug is sufficient to inhibit IKK-beta and therefore activation of NF- κ B or to achieve any other therapeutic indication as disclosed herein. Typically, a pharmaceutical composition containing the compound is administered in a topical formulation of between about 0.01% to about
10 5% w/w.

No unacceptable toxicological effects are expected when the compound of the present invention is administered in accordance with the present invention.

The ability of the compound described herein to inhibit the activation of NF- κ B is clearly evidenced in its ability to inhibit the phosphorylation of the N-terminal
15 fragment of I κ B- α by IKK- β . This compound also blocks the degradation of I κ B- α and the nuclear translocation of NF- κ B in human monocytes and other mammalian cells upon activation of the cells with a pro-inflammatory stimuli (e.g., TNF- α , LPS, etc.). In addition this compound inhibits pro-inflammatory mediator production from LPS-stimulated human monocytes and stimulated human primary synovial
20 fibroblasts. The utility of the present NF- κ B inhibitor in the therapy of diseases is premised on the importance of NF- κ B activation in a variety of diseases. In addition, the compound of Formula I has been shown to be orally bioavailable in preclinical species such as rat and mouse. The in vivo profile of the compound of Formula I displayed significantly improved DMPK profile in the preclinical species
25 tested than the analog lacking the 4'-F group while retaining comparable IKK-2 inhibitory activity.

NF- κ B plays a key role in the regulated expression of a large number of pro-inflammatory mediators including cytokines such as TNF, IL-1 β , IL-6 and IL-8 (Mukaida *et al.*, 1990; Liberman and Baltimore, 1990; Matsusaka *et al.*, 1993), cell
30 adhesion molecules, such as ICAM and VCAM (Marui *et al.*, 1993; Kawai *et al.*, 1995; Ledebur and Parks, 1995), and inducible nitric oxide synthase (iNOS) (Xie *et*

al., 1994; Adcock *et al.*, 1994). (Full reference citations are at the end of this section). Such mediators are known to play a role in the recruitment of leukocytes at sites of inflammation and in the case of iNOS, may lead to organ destruction in some inflammatory and autoimmune diseases (McCartney-Francis *et al.*, 1993; Kleemann
5 *et al.*, 1993).

Evidence for an important role of NF- κ B in inflammatory disorders is obtained in studies of asthmatic patients. Bronchial biopsies taken from mild atopic asthmatics show significant increases in the number of cells in the submucosa staining for activated NF- κ B, total NF- κ B, and NF- κ B-regulated cytokines such as
10 GM-CSF and TNF α compared to biopsies from normal non-atopic controls (Wilson *et al.*, 1998). Furthermore, the percentage of vessels expressing NF- κ B immunoreactivity is increased as is IL-8 immunoreactivity in the epithelium of the biopsy specimens (Wilson *et al.*, 1998). As such, inhibition of IL-8 production through the inhibition of NF- κ B, as has been demonstrated by this compound would
15 be predicted to be beneficial in airway inflammation.

Recent studies suggest that NF- κ B may also play a critical role in the pathogenesis of inflammatory bowel disease (IBD). Activated NF- κ B is seen in colonic biopsy specimens from Chron's disease and ulcerative colitis patients (Ardite *et al.*, 1998; Rogler *et al.*, 1998; Schreiber *et al.*, 1998). Activation is evident in the
20 inflamed mucosa but not in uninflamed mucosa (Ardite *et al.*, 1998; Rogler *et al.*, 1998) and is associated with increased IL-8 mRNA expression in the same sites (Ardite *et al.*, 1998). Furthermore, corticosteroid treatment strongly inhibits intestinal NF- κ B activation and reduces colonic inflammation (Ardite *et al.*, 1998; Schreiber *et al.*, 1998). Again, inhibition of IL-8 production through the inhibition
25 of NF- κ B, as has been demonstrated by this compound would be predicted to be beneficial in inflammatory bowel disease.

Animal models of gastrointestinal inflammation provide further support for NF- κ B as a key regulator of colonic inflammation. Increased NF- κ B activity is observed in the lamina propria macrophages in 2,4,6,-trinitrobenzene sulfonic acid
30 (TNBS)-induced colitis in mice with p65 being a major component of the activated

complexes (Neurath *et al.*, 1996; Neurath and Pettersson, 1997). Local administration of p65 antisense abrogates the signs of established colitis in the treated animals with no signs of toxicity (Neurath *et al.*, 1996; Neurath and Pettersson, 1997). As such, one would predict that small molecule inhibitors of NF- κ B would be useful in the treatment of IBD.

Further evidence for a role of NF- κ B in inflammatory disorders comes from studies of rheumatoid synovium. Although NF- κ B is normally present as an inactive cytoplasmic complex, recent immunohistochemical studies have indicated that NF- κ B is present in the nuclei, and hence active, in the cells comprising human rheumatoid synovium (Handel *et al.*, 1995; Marok *et al.*, 1996; Sioud *et al.*, 1998) and in animal models of the disease (Tsao *et al.*, 1997). The staining is associated with type A synoviocytes and vascular endothelium (Marok *et al.*, 1996). Furthermore, constitutive activation of NF- κ B is seen in cultured synoviocytes (Roshak *et al.*, 1996; Miyazawa *et al.*, 1998) and in synovial cell cultures stimulated with IL-1 β or TNF α (Roshak *et al.*, 1996; Fujisawa *et al.*, 1996; Roshak *et al.*, 1997). Thus, the activation of NF- κ B may underlie the increased cytokine production and leukocyte infiltration characteristic of inflamed synovium. The ability of this compound to inhibit NF- κ B and thereby inhibit the production of pro-inflammatory mediators (e.g. cytokines and prostanoids) by these cells would be predicted to yield benefit in rheumatoid arthritis.

Biological Assays:

The compound of this invention may be tested in one of several biological assays to determine the concentration of compound, which is required to have a given pharmacological effect.

NF- κ B activity may also be measured in an electrophoretic mobility shift assay (EMSA) to assess the presence of NF- κ B protein in the nucleus. The cells of interest are cultured to a density of 1×10^6 /mL. The cells are harvested by centrifugation, washed in PBS without Ca²⁺ and Mg²⁺ and resuspended in PBS with Ca²⁺ and Mg²⁺ at 1×10^7 cells/mL. To examine the effect of compound on the

activation of NF- κ B, the cell suspensions are treated with various concentrations of drug or vehicle (DMSO, 0.1%) for 30 min. at 37 °C prior to stimulation with TNF- α (5.0 ng/mL) for an additional 15 min. Cellular and nuclear extracts are prepared follows. Briefly, at the end of the incubation period the cells (1×10^7 cells) are
5 washed 2x in PBS without Ca^{2+} and Mg^{2+} . The resulting cell pellets are resuspended in 20 μL of Buffer A (10 mM Hepes (pH 7.9), 10 mM KCl, 1.5 mM MgCl_2 , 0.5 mM dithiothreitol (DTT) and 0.1% NP-40) and incubated on ice for 10 min. The nuclei are pelleted by microcentrifugation at 3500 rpm for 10 min at 4 °C. The resulting supernatant was collected as the cellular extract and the nuclear pellet was
10 resuspended in 15 μL Buffer C (20 mM Hepes (pH 7.9), 0.42 M NaCl, 1.5mM MgCl_2 , 25% glycerol, 0.2 mM EDTA, 0.5 mM DTT, and 0.5 mM phenylmethylsulphonyl fluoride (PMSF)). The suspensions are mixed gently for 20 min at 4 °C then microcentrifuged at 14,000 rpm for 10 min at 4 °C. The supernatant is collected and diluted to 60 μL with Buffer D (20mM Hepes (pH 7.9),
15 50 mM KCl, 20% glycerol, 0.2 mM EDTA, 0.5 mM DTT, and 0.5 mM PMSF). All samples are stored at -80 °C until analyzed. The protein concentration of the extracts is determined according to the method of Bradford (Bradford, 1976) with BioRad reagents.

The effect of compounds on transcription factor activation is assessed in an
20 electrophoretic mobility shift assay (EMSA) using nuclear extracts from treated cells as described above. The double stranded NF- κ B consensus oligonucleotides (5'-AGTTGAGGGGACTTTCCCAGGC-3') are labelled with T_4 polynucleotide kinase and [g^{-32}P]ATP. The binding mixture (25 μL) contains 10 mM Hepes-NaOH (pH 7.9), 4 mM Tris-HCl (pH 7.9), 60 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 10%
25 glycerol, 0.3 mg/mL bovine serum albumin, and 1 μg poly(dI-dC)•poly(dI-dC). The binding mixtures (10 μg nuclear extract protein) are incubated for 20 min at room temperature with 0.5 ng of ^{32}P -labelled oligonucleotide (50,000-100,000 cpm) in the presence or absence of unlabeled competitor after which the mixture is loaded on a 4% polyacrylamide gel prepared in 1X Tris borate/EDTA and electrophoresed at 200
30 V for 2 h. Following electrophoresis the gels are dried and exposed to film for detection of the binding reaction.

The effect of compounds on the phosphorylation of I κ B may be monitored in a Western blot. Cellular extracts are subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels (BioRad, Hercules, CA) and the proteins transferred to nitrocellulose sheets (Hybondtm-ECL, Amersham Corp., Arlington Heights, IL). Immunoblot assays are performed using a polyclonal rabbit antibody directed against I κ B α or I κ B β followed with a peroxidase-conjugated donkey anti-rabbit secondary antibody (Amersham Corp., Arlington Heights, IL). Immunoreactive bands are detected using the Enhanced Chemiluminescence (ECL) assay system (Amersham Corp., Arlington Heights, IL).

Assays for I κ B kinases were conducted as follows: IKK- α was expressed as a hexa-histidine tagged protein in baculovirus-infected insect cells and purified over a Ni-NTA affinity column. Kinase activity was assayed using 50 ng of purified protein in assay buffer (20 mM Hepes, pH 7.7, 2 mM MgCl₂, 1 mM MnCl₂, 10 mM β -glycerophosphate, 10 mM NaF, 10 mM PNPP, 0.3 mM Na₃VO₄, 1 mM benzamidine, 2 μ M PMSF, 10 μ g/ml aprotinin, 1 μ g/mL leupeptin, 1 μ g/mL pepstatin, 1mM DTT) containing various concentrations of compound or DMSO vehicle and ATP as indicated (Pharmacia Biotech Inc., Piscataway, NJ). The reaction was started by the addition of 200 ng I κ B-GST (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), in a total volume of 50 μ L. The reaction was allowed to proceed for 1 h. at 30 °C after which the reaction was terminated by the addition of EDTA to a final concentration of 20 mM. Kinase activity was determined by dissociation-enhanced lanthanide fluorescence immunoassay (Wallac Oy, Turku, Finland) using a phospho-I κ B- α (Ser32) antibody (New England Biolabs, Inc., Beverly, MA) and an Eu³⁺-labelled anti-rabbit IgG (Wallac Oy, Turku, Finland). The plates were read in a VICTOR 1420 Multilabel Counter (Wallac), using a standard europium protocol (excitation 340 nm, emission 615 nm; fluorescence measured for 400 μ s after a 400 usec delay). Data are expressed as fluorescence (cps) units.

IKK- β was expressed as a GST-tagged protein, and its activity was assessed in a 96-well scintillation proximity assay (SPA). Briefly, IKK- β was diluted in assay

buffer as described above (20 nM final), with various concentrations of compound or DMSO vehicle, 240 nM ATP and 200 nCi [γ - 33 P]-ATP (10 mCi/mL, 2000 Ci/mmol; NEN Life Science Products, Boston, MA). The reaction was started with the addition of a biotinylated peptide comprising amino acids 15 – 46 of I κ B- α (American Peptide) to a final concentration of 2.4 μ M, in a total volume of 50 μ L. The sample incubated for one hour at 30 °C, followed by the addition of 150 μ L of stop buffer (PBS w/o Ca $^{2+}$, Mg $^{2+}$, 0.1% Triton X-100 (v/v), 10 mM EDTA) containing 0.2 mg streptavidin-coated SPA PVT beads (Amersham Pharmacia Biotech, Piscataway, NJ). The sample was mixed, incubated for 10 min. at room temperature, centrifuged (1000 xg, 2 minutes), and measured on a Hewlett-Packard TopCount.

In addition, IKK- β or IKK- α activity is measured by phosphorylation of recombinant GST-I κ B α using time-resolved fluorescence resonance energy transfer (TR-FRET) in 384-well microtitre plates. Briefly IKK- β or IKK- α is diluted in assay buffer (50 mM HEPES pH 7.4 containing 10 mM magnesium chloride, 1 mM CHAPS, 1 mM DTT and 0.01% w/v BSA) to 5 nM final concentration. This is added to various concentrations of compound or DMSO vehicle and the reaction started by addition of 25 nM GST-I κ B α and 1 μ M ATP in assay buffer to a volume of 30 μ L. After incubation for 30 min at ambient temperature the reaction was stopped by addition of 50 mM pH 7.4 EDTA (15 μ L). Detection of phosphorylated product was achieved by addition of a LANCE europium chelate labelled specific anti-phosphoserine monoclonal antibody at 0.5 nM final concentration (Cell signalling Technology via Perkin Elmer) and allophycocyanin labelled anti-GST antibody at 10 nM final concentration (Prozyme) to give a final volume of 60 μ L. After a further incubation at ambient temperature of a least 30 min the signal was read on a Perkin Elmer Discovery fluorimeter.

The effect of IKK- β inhibitors on primary synovial fibroblast mediator production was assessed as follows: Primary cultures of human RSF were obtained by enzymatic digestion of synovium obtained from adult patients with rheumatoid arthritis as previously described (Roshak *et al.*, 1996b). Cells were cultured in Earl's Minimal Essential Medium (EMEM) which contained 10% fetal bovine serum

(FBS), 100 units/ml penicillin and 100 µg/ml streptomycin (GIBCO, Grand Island, NY), at 37°C and 5% CO₂. Cultures were used at passages 4 through 9 in order to obtain a more uniform type B fibroblast population. For some studies, fibroblasts were plated at 5 x 10⁴ cells/mL in 16 mm (diameter) 24 well plates (Costar, Cambridge, MA). Cells (70-80% confluence) were exposed to IL-1β (1 ng/mL) (Genzyme, Cambridge, MA) for the designated time. Drugs in DMSO vehicle (1%) were added to the cell cultures 15 minutes prior to the addition of IL-1. Studies were conducted 3-4 times using synovial cells from different donors. RSF cellular extracts were prepared from cells treated as described above. Briefly, human RSF were removed by trypsin/EDTA, washed, and harvested by centrifugation. Cellular extracts were prepared as previously described (Dignam *et al.*, 1983; Osborn, *et al.*, 1989). Briefly, at the end of the incubation period the cells (1x10⁷ cells) were washed 2x in PBS without Ca²⁺ and Mg²⁺. The resulting cell pellets were resuspended in 20 µL of Buffer A (10 mM Hepes (pH 7.9), 10 mM KCl, 1.5 mM MgCl₂, 0.5 mM.

Effect of IKK-β inhibition on human monocyte stimulated eicosanoid and cytokine production was assessed as follows: Monocytes were isolated from heparinized whole blood by double gradient centrifugation as previously described. Isolated monocyte enriched PBMCs were then adhered to 24 well culture plates at 2 x 10⁶ cells/mL in RPMI 1640 10% FBS (Hyclone, Logan, Utah) for 2 h. to further enrich the monocyte population. The media was then removed, cells washed once with RPMI 1640, and 1 mL RPMI 1640 10% FBS was added to the wells. Test compounds are added to the wells with a final vehicle concentration of 0.05% DMSO. Monocytes were activated by the addition of 200 ng/mL endotoxin (LPS; *E. coli* serotype 026:B6)(Sigma, St. Louis, MO.) and incubated for 24 hrs. Cell-free supernates were analyzed by ELISA for TNF-α (EIA developed at SB), PGE₂ (Cayman Chemical, Ann Arbor, MI), and IL-8 and IL-6 Biosource International, Camarillo, CA). Viability of the cells was determined by trypan blue exclusion.

Effect of IKK-β inhibitors on phorbol ester-induced inflammation was assessed as follows: The inflammatory response induced by the cutaneous application of phorbol ester (PMA) to the external pinnae of Balb/c mice has proven

to be a useful model to examine multifactorial inflammatory cell infiltration and inflammatory alteration of epidermis. The intense inflammatory lesion is dominated by neutrophil infiltration, which can be easily quantified by measurement tissue concentration myeloperoxidase, an azuriphilic granular enzyme present in neutrophils. In addition, the overall intensity of the inflammatory response can be measured by determination of ear thickness. Balb/c mice (n = 6/group) were administered drug treatment or vehicle followed by PMA (4 ug/ear). The mice were sacrificed 4 h. later, the ear thickness determined and NF- κ B activation was monitored by I κ B α western or EMSA analysis.

Effect of IKK- β inhibitors on rat carrageenan-induced paw edema was assessed as follows: Male Lewis rats (Charles River- Raleigh, NC) were housed and allowed free access to food and water, and weighed between 200-275g for each experiment. Compound or vehicle (0.5% Tragacanth (p.o.) or 10%DMSO, 5%DMA, 30% Cremophor(i.p.)) was administered 30 minutes to 1 hour prior to the carrageenan injection. Edema was induced by injection of 1% carrageenan in sterile dH₂O (0.05ml/paw) into the plantar surface of the right hindpaw. Paw thickness was measured prior to administration of compound or vehicle, and again at 3 hours, to determine change in paw volume. Rats were euthanized by CO₂ inhalation and the right hindfoot was removed, immediately frozen in liquid nitrogen and stored at -80C for analysis.

To determine the effects of an IKK-2 inhibitor in the mouse collagen-induced arthritis (CIA) model, 12 male DBA/1 mice (20-22 grams) per treatment group were immunized on day 0 with a total of 100 uL of complete Freund's adjuvant (CFA) containing 200 ug of bovine type II collagen. On day 21 mice were boosted with 100 uL of phosphate buffered saline (PBS) containing 200 ug of bovine type II collagen (the 100 uL of collagen/CFA or collagen/PBS was injected subcutaneously into the tail). The IKK-2 inhibitor in vehicle, or vehicle alone, was administered intraperitoneally, twice daily, from days 1 through 40 (disease symptoms are evident beginning on days 25-28). Two additional treatment groups included the positive control etanercept (Enbrel) (4 mg/kg, intraperitoneally, every other day), and the etanercept vehicle (PBS). Mice were scored daily, through day 50, for clinical

symptoms (see below), and paw thicknesses were measured. In addition to the 12 mice per treatment group that were scored throughout the experiment, at several time points during the course of disease satellite mice (3-5 per treatment group) treated as described above were utilized to measure cytokine/chemokine levels and p65 levels in the paw, the *ex vivo* antigen recall response by draining lymph node antigen recall response by draining lymph node

Induction of arthritis AIA is induced by a single injection of 0.75 mg of *Mycobacterium butyricum* (Difco, Detroit, MI) suspended in paraffin oil into the base of the tail of male Lewis rats aged 6-8 weeks (160-180 g). Hindpaw volumes are measured by a water displacement method on day 16 and/or day 20. Test compounds were homogenized in a suitable vehicle and administered by a suitable route. Control animals are administered vehicles alone. Two dosing protocols are generally used: prophylactic dosing, which is initiated on the day of adjuvant injection and therapeutic administration, initiated on day 10 once inflammation has been established.

Clinical scoring

Each paw was assigned a score ranging from 0-4, based on the following criteria:

0 = no inflammation

1 = single swollen digit

2 = several swollen digits, mild paw swelling

3 = several swollen digits, moderate paw swelling

4 = all digits swollen, severe paw swelling

General

Nuclear magnetic resonance spectra were recorded at either 250, 300 or 400 MHz using, respectively, a Bruker AM 250, Bruker ARX 300 or Bruker AC 400 spectrometer. CDCl₃ is deuteriochloroform, DMSO-d₆ is hexadeuteriodimethylsulfoxide, and CD₃OD is tetradeuteriomethanol. Chemical shifts are reported in parts per million (δ) downfield from the internal standard tetramethylsilane. Abbreviations for NMR data are as follows: s = singlet, d =

doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, app = apparent, br = broad. J indicates the NMR coupling constant measured in Hertz. Continuous wave infrared (IR) spectra were recorded on a Perkin-Elmer 683 infrared spectrometer, and Fourier transform infrared (FTIR) spectra were recorded on a Nicolet Impact 400 D infrared spectrometer. IR and FTIR spectra were recorded in transmission mode, and band positions are reported in inverse wavenumbers (cm^{-1}). Mass spectra were taken on either VG 70 FE, PE Syx API III, or VG ZAB HF instruments, using fast atom bombardment (FAB) or electrospray (ES) ionization techniques. Elemental analyses were obtained using a Perkin-Elmer 240C elemental analyzer. Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. All temperatures are reported in degrees Celsius.

Analtech Silica Gel GF and E. Merck Silica Gel 60 F-254 thin layer plates were used for thin layer chromatography. Both flash and gravity chromatography were carried out on E. Merck Kieselgel 60 (230-400 mesh) silica gel.

Where indicated, certain of the materials were purchased from the Aldrich Chemical Co., Milwaukee, Wisconsin, TCI America, Portland, OR..

Examples

In the following synthetic example, temperature is in degrees Centigrade ($^{\circ}\text{C}$). Unless otherwise indicated, all of the starting materials were obtained from commercial sources. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. This example is given to illustrate the invention, not to limit its scope. Reference is made to the claims for what is reserved to the inventors hereunder.

25

Example 1

Preparation of 5-(4-Fluoro-phenyl)-2-ureido-thiophene-3-carboxylic acid amide

a. Preparation of (4-Fluoro-phenyl)-acetaldehyde

To a stirring solution of 4-fluorophenylethyl alcohol (110 g, 184.8 mmol) in dry DMSO (800 mL) triethylamine (220 mL, 1589.2 mmol) was added dropwise. The temperature was maintained at 15 $^{\circ}\text{C}$ stirring under N_2 . A solution of pyridine

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sulfur trioxide (253 g, 1589.2 mmol) in dry DMSO (900 mL) was added dropwise to the reaction keeping the temperature below 25 °C. The subsequent mixture was stirred at 15 °C for 1.5 h. After this time, 1N HCl (aqueous) was added carefully until the mixture reached pH 4.3. The reaction mixture was extracted with MTBE,
5 the organic layer dried and evaporated to give 4-fluorophenylacetaldehyde (110.8 g) as yellow oil.

b. Preparation of 2-Amino-5-(4-fluoro-phenyl)-thiophene-3-carboxylic acid amide

In a procedure similar to the one described by A.C. Goudie, US Patent
10 3,963,750, a solution of 4-fluorophenylacetaldehyde (110.8 g) in dry DMF (350 mL) was treated with 2-cyanoacetamide (65.9 g, 784.8 mmol) and sulfur (25.2 g, 784.8 mmol). Triethyl amine (79.4 g, 784.8 mmol,) was added dropwise to the reaction using an ice bath to control the resulting exotherm. The reaction was stirred at room temperature for 22 h (overnight). The reaction mixture was then poured into ice
15 water and ethyl acetate added. An emulsion formed and the insoluble material at the interface was filtered and dried overnight to give 2-amino-5- (4-fluoro-phenyl)-thiophene-3-carboxylic acid amide yellow solid (77.4 g). LC MS [M+H]⁺ m/e 237.

c. 5-(4-Fluoro-phenyl)-2-ureido-thiophene-3-carboxylic acid amide

To a suspension of 2-amino-5-(4-fluoro-phenyl)-thiophene-3-carboxylic acid
20 amide (77.4 g, 327.5 mmol) in dichloromethane (1,300 mL) at 0 °C was added chlorosulfonylisocyanate (47.7 g, 337 mmol) dropwise over 0.5 h keeping the temperature below 5 °C. After stirring the mixture for 1 h at 0 °C, the temperature was raised to room temperature (hplc analysis showed complete reaction). Water
25 (800 mL) was added (temperature kept below 20 °C) and the solid filtered and dried under vacuum at 30 °C. Recrystallization from ethanol gave 64.3 g of 5-(4-fluoro-phenyl)-2-ureido-thiophene-3-carboxylic acid amide. LC MS [M+H]⁺ m/e 280.

All publications, including but not limited to patents and patent
applications, cited in this specification are herein incorporated by reference as if
30 each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims.

Without further elaboration, it is believed that one skilled in the area can, using

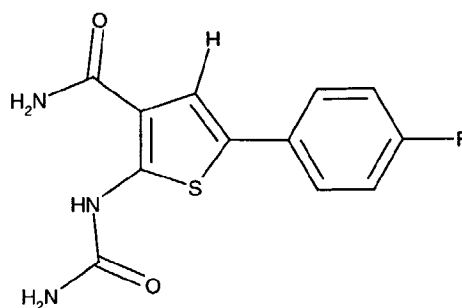
5 the preceding description, utilize the present invention to its fullest extent.

Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

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What is claimed is:

1. 5-(4-fluoro-phenyl)-2-ureido-thiophene-3-carboxylic acid amide, also having the structure:



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2. A method of treating a disease characterized by pathological NF- κ B activation comprising inhibiting the pathological activation by administering to a patient in need thereof an effective amount of a compound according to claim 1.
- 10 3. A method according to claim 2 wherein the disease is an inflammatory or tissue repair disorder.
4. A method according to claim 3 wherein the disease is selected from the group consisting of inflammatory and tissue repair disorders, particularly rheumatoid
 - 15 arthritis, inflammatory bowel disease, asthma and COPD (chronic obstructive pulmonary disease) osteoarthritis, osteoporosis and fibrotic diseases, dermatosis, including psoriasis, atopic dermatitis and ultraviolet radiation (UV)-induced skin damage, autoimmune diseases including systemic lupus erythematosus, multiple sclerosis, psoriatic arthritis, ankylosing spondylitis, tissue and organ rejection,
 - 20 Alzheimer's disease, stroke, atherosclerosis, restenosis, diabetes, glomerulonephritis, cancer, including Hodgkins disease, cachexia, inflammation associated with infection and certain viral infections, including acquired immune deficiency syndrome (AIDS), adult respiratory distress syndrome, and Ataxia Telangiectasia.
- 25 5. A method according to claim 3 wherein said disease is dermatosis.

6. A method according to claim 3 wherein the disease is selected from the group consisting of: psoriasis, atopic dermatitis, and UV-induced skin damage.
7. A method according to claim 3 wherein the disease is selected from the group consisting of autoimmune diseases; tissue and organ rejection, Alzheimer's disease, stroke, atherosclerosis, restenosis, diabetes, glomerulonephritis, osteoarthritis, osteoporosis, and Ataxia Telangiectasia.
8. A method according to claim 3 wherein said disease is an autoimmune disease.
9. A method according to claim 3 wherein the autoimmune disease is systemic lupus erythematosus, multiple sclerosis, psoriatic arthritis, or ankylosing spondylitis, diabetes
10. A method according to claim 3 wherein the disease is cancer and or cachexia.
11. A method according to claim 3 wherein the cancer is Hodgkins disease.
12. A method according to claim 3 wherein the disease is inflammation associated with infection and certain viral infections, including acquired immune deficiency syndrome (AIDS).
13. A method according to claim 3 wherein the disease is AIDS.
14. A method according to claim 3 wherein the disease is adult respiratory distress syndrome.
15. A method according to claim 3 wherein said disease is COPD.
16. A method according to claim 3 wherein said disease is asthma.

17. A method according to claim 3 wherein said disease is rheumatoid arthritis.
18. A method according to claim 3 wherein there is dual inhibition of NF- κ B and checkpoint kinase.

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